A COMPARATIVE STUDY OF SOME DYSENTERY BACILLI.

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Before discussing the main theme of this article, it may be convenient to review briefly modern views regarding the classification of dysentery. It is doubtful whether the literature of any disease is more encumbered with a mass of names, indicating the nature of the disorder or the author’s conception of its pathology, than is that of dysentery. On this account it is probable that, under the term dysentery, there may have been included in the past, and even be still included, more than one pathological entity. Limiting our conception of the affection to that of a disease marked by frequent bloody and mucous stools, fever, tenesmus and abdominal pain in different degrees, coupled with thickening of the walls, hemorrhages and ulceration of the mucous and submucous coats of the large intestine, notably of the cecum and rectum, we find that the group of symptoms and lesions known as dysentery are conveniently classified into the following, namely: the endemic, the epidemic, and the sporadic classes.

For this division of the idiopathic dysenteries we are mainly indebted to Kartulis, who specially distinguishes the endemic or tropical form of dysentery by its climatic and geographical peculiarities and its pathological anatomy, claiming an amoeba as its constant cause. With this general classification most authorities tacitly agree, subject, however, to the proviso that although an amoeba may be the cause, at least, of an Egyptian endemic or tropical dysentery, yet the origin of epidemic dysenteries is essentially bacillary. As for the so-called sporadic forms, these are caused by chemical or mechanical means, or by the presence of parasites. A very superficial knowledge of the disease shows that the different forms of dysentery are clinically indistinguishable, since all have catarrhal, ulcerated and diphtheritic stages, accompanied by such symptoms as colic, tenesmus, slimy bloody stools, and later fever, rigors, loss of appetite and exhaustion. The so-called endemic dysentery has no tendency to spontaneous cure and is mainly chronic and
ulcerative, affecting the caecum and ascending colon, while the epidemic type is of shorter duration, catarrhal and ulcerative, involving the whole colon and rectum. Whether we consider the disease from the clinical, etiological, or geographical point of view, it is clear that the pathological anatomy and its exciting cause constitute the real differences between the types or classes. Under these circumstances there appears to be no reason why we should not drop the terms endemic and epidemic, but adopt, as the basis of classification, the sole difference between the two types, namely, the presence of amoebae or of apparently causative bacilli.

Whatever may be the pathological differences between amoebic and bacillary dysentery, they agree in their independence of dampness of soil or climate and of variations in temperature, also in their greater incidence in hot weather and under circumstances of poverty and bad or fouled water supply. Although it is not the purpose of this article to discuss the causative agent of amoebic or so-called endemic dysentery, it may be stated that Kartulis' case in support of his plea that amoebae are the cause of all endemic dysenteries is a strong one, but until these amoebae can be cultivated outside the body and then shown to be capable of causing a typical dysentery with amoebic stools in infected animals, the problem cannot be considered as solved absolutely. Leaving this aspect of the dysenteric question, we may now pass to a consideration of the various organisms which have been described as the cause of epidemic-non-amoebic or bacillary dysentery.

The presence of bacteria in the stools and tissues in epidemic dysentery has been known for some years, but the credit of first recognising a definite pathogenic bacillus in that disease rests with Chantemesse and Widal, who described its characters and its pathogenic action on animals. Certain researches by Kruse and Pasquale and by Celli and Fiocca, on the same lines, were to some extent confirmative. These latter investigators considered it highly probable that the disease was caused by a bacillus closely allied to the _B. coli_, which they had isolated from dysenteric cases. With cultures of this bacillus they claimed to produce dysentery in cats, and a toxin separated from growths of the organism was found to give rise to similar conditions. Identical results were obtained by del Pino and Aless-
andri, while Escherich also expressed his belief in the important part played by certain highly virulent bacilli allied to the coli group which he had isolated from a contagious enteric disorder in children whose morbid anatomy agreed closely with catarrhal dysentery. On the other hand, a causative rôle in the production of dysentery had also been ascribed to the pyogenic cocci, especially the streptococci, which are constantly found in association with the intestinal bacilli. The chief exponent of this view has been Zancarol, whose studies have been endorsed to some extent by those of Silvestri in Turin, and of Ascher who investigated cases of the kind in Prussia. The cocci isolated by Ascher were said to have shown the agglutination reaction with the blood serum of the patients from whom they were obtained. In the same way a variety of endemic dysentery prevailing in Cochin China was believed by Calmette to be due to the \textit{B. pyocyaneus}, while the same micro-organism was isolated from a small epidemic of the disease occurring in America by Lartigau, and in Canada by Adami.

From the foregoing summary, it is clear that there has been no lack of variety in micro-organisms to which the causation of dysentery has been attributed. Much of the experimental work of these observers was carried out at a time and under conditions when bacteriological methods were more imperfect than they are now. For this reason too great importance must not be attached to their conclusions, particularly as subsequent researches have shown that the bacilli so far enumerated, except those of Chantemesse and Widal, possess doubtful specific properties. The investigations of an epidemic of dysentery in Japan during 1897, by Shiga, have yielded somewhat different and apparently more convincing results, inasmuch as he not only isolated a special bacillus from the stools of the affected, but showed its specificity by demonstrating the agglutination of its cultures by blood serum. It is true his experiments upon animals failed to reproduce the symptoms and intestinal lesions so characteristic of human dysentery; still, relying on the constant presence of the bacillus in the excreta of those suffering from dysentery, its absence from the discharges of other sick persons, and its agglutination by the serum of the affected, Shiga believed that he had isolated the probable cause of epidemic dysentery, or at least of that form...
which prevails in Japan. Two years later, Kruse discovered an apparently identical bacillus in an epidemic of dysentery which prevailed in Rhenish Westphalia. At much the same time Flexner, in the Philippines, isolated from certain persons, suffering from dysentery, a bacillus presenting close resemblances to that obtained by Shiga in Japan. Flexner's work was practically confirmed soon after by Strong and Musgrave in their study of dysentery among the American troops in Manilla, while Drigalski, in an outbreak among the Prussian Guards encamped at Doberitz, Pfuhl, among men returned from the China expedition, and Müller, in an epidemic at Sudsteiernark, all describe an identical bacillus. Vedder and Duval recovered in several outbreaks of dysentery in the United States bacilli analogous to those isolated by Flexner in Manilla and Porto Rico; so, too, Rosenthal in Moscow reports to the same effect, while Rogers has found a bacillus closely resembling that described by Shiga in the stools of catarrhal dysentery in India. To the same effect are the observations of Park and Carey, made during an extensive outbreak in Tuckahoe, New York State. More recently Vaillard and Dopter give details of a micro-organism isolated by them in an epidemic of dysentery in the garrison of Vincennes, which they consider to be the same as that noted by the other observers. In short, from the most diverse parts of the globe has accumulated a considerable mass of evidence indicating that there exists an epidemic form of dysentery, characterised by a special micro-organism whose constant presence in the intestinal discharges and agglutination by the blood serum of those affected suggests its being considered as the probable cause of this disease.

The morphological and cultural characters of this micro-organism may be thus described. It is a short thin rod, varying from 1 to 3 μ in length and a trifle thicker than the Bacillus typhosus. Its ends are rounded, is non-motile, apparently without cilia, never forms spores, easily stains with the ordinary aniline dyes, but decolourises by Gram's method of staining. Bi-polar staining and the appearance of involution forms are not uncommon. It grows well at 37° C. on all the ordinary cultural media, but preferably in the presence of oxygen. Both as to motility and the presence of cilia some difference of opinion is evident in the literature concerning this bacillus.
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As the result of a somewhat extended study of this microorganism, derived from a variety of sources, I am unable to affirm that it is either motile or possessed of cilia.

On a gelatine slope the bacillus of dysentery grows as a fine pearly pellicle with slightly serrated margins, not unlike that given by the *B. typhosus*. In plates made of this medium, the surface colonies are delicate pearl grey expansions, more or less translucent, with waved or indented margins and marked by delicate furrows and ridges suggestive of the finer venations on a vine leaf. The gelatine is not liquefied. In the accompanying Plate I. are shown typical colonies of some varieties of this bacillus. In all these features it presents many similarities to colonies of the enteric fever organism. It, however, grows a trifle more rapidly on gelatine than does this latter bacillus, but distinctly more slowly than does the *B. coli communis*. In fact, not only in its behaviour and mode of growth on gelatine, but also in other media, the dysentery microorganism suggests an intermediate type between the enteric fever and the common colon bacillus. Its colonies in the gelatine depth present no distinctive characteristics.

Upon ordinary sloped agar the growth is moist, grey, slightly opalescent, and very much like that of the *B. typhosus*. If the agar be tinted with neutral red in the presence of either glucose or lactose, subsequent inoculation with the *B. dysenteriae* causes no change of colour.

In peptone broth the micro-organism causes a uniform turbidity, without the formation of any pellicle. After a few days, usually about the third day, a flocculent deposit forms and the reaction of the medium becomes slightly acid. The majority of the strains of this bacillus which I have examined fail to produce indol either in peptone bouillon or in peptone and salt solution. As will be seen from details to be given subsequently, there are some apparent exceptions to this rule. On the other hand, all varieties reduce nitrates. The growth on potato is somewhat variable, but in the majority of cases it is colourless, moist and glistening, while in some varieties it is of a pale yellow to a light brown colour.

In milk, the various dysentery bacilli grow well, producing no clot. The reaction of this medium does not sensibly alter for the first four days, but after that period there may be
PLATE I.

Colony of B. Dysent. (Shiga III.), 72 hrs. × 30.

Colony of B. Dysent. (Flexner III.), 72 hrs. × 30.

Colony of B. Dysent. (Kruse II.), 72 hrs. × 30.

Colony of B. Dysent. (Vaillard), 72 hrs. × 30.

Colony of B. Dysent. (Cray), 72 hrs. × 30.

Colony of B. Dysent. (Bruce G.), 72 hrs. × 30.

Illustrating paper by Lieut.-Col. Firth.
some production of a faint acidity, but passing always to
definite alkalinity about the eighth or tenth day.

When grown in the various sugars there is no production of
gas by this bacillus, but all strains cause a small production
of acid in glucose, and a few varieties give rise to acid
production in mannite, maltose and galactose. Starch and
glycerine are quite unaffected. Its behaviour in the media
suggested by Proskauer and Capaldi is similar, on the whole,
to that shown by the enteric bacillus. Practically all varieties
fail to grow in No. 1 medium, while in No. 2 their growth
results in either no change in reaction, or, at most, a faint
acidity.

The accompanying table gives in some detail the chief cul­
tural characteristics of the different strains of the dysentery
bacillus which have been examined by me. Two of these were
received from Dr. Flexner and are shown as Flexner I. and II.;
Flexner III. came from Dr. Kral, of Prague, and Flexner IV.
from Dr. MacConkey. Shiga I. came also from Dr. MacConkey,
Shiga II. came from Dr. Kral, and Shiga III. from Dr. Bulloch.
The varieties called Harris and Gray came through Dr. Mac­
Conkey, and were, I believe, originally obtained in Manilla by
Flexner; while Pickering and Landon were obtained by myself
from the stools of two soldiers at Netley, who had been
invalided with dysentery from India and South Africa respec­
tively. The Kruse I. came from Dr. Kral, and that marked
II. from Dr. Flexner, while the variety marked Bruce G. was
given me by Lieut.-Col. Bruce and obtained by him from a
case of dysentery in South Africa. Col. Bruce made no claims
for it being regarded as the causative agent of South African
dysentery; it was the only strain surviving, on his return, of
a number of micro-organisms which he isolated from dysentery
cases, and is included in this comparative study as one of a
series of closely allied bacteria intimately associated with that
disease. The variety called Vaillard was sent to me by Dr.
Vaillard, of Vincennes, having been isolated in the outbreak of
dysentery in that garrison already referred to.

For the sake of brevity the letters N.G. are used to signify
the fact of no gas production. Further, where a statement is
made that a given percentage of acid was produced, the per­
centage is in terms of decinormal sulphuric acid, the degree of
### Cultural Characteristics of Some Varieties of Dysentery Bacilli

<table>
<thead>
<tr>
<th>Description</th>
<th>Flexner I</th>
<th>Flexner II</th>
<th>Flexner III</th>
<th>Flexner IV</th>
<th>Harris</th>
<th>Gray</th>
<th>Fickett, Ind.</th>
<th>Landen</th>
<th>Bridge, G.</th>
<th>Shiga, L. &amp; H.</th>
<th>Krebs, L. &amp; H.</th>
<th>Vajlard</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphology</strong></td>
<td>Small non-motile rod, often in pairs</td>
<td>Small non-motile rod, often in pairs</td>
<td>Small non-motile rod, often in pairs</td>
<td>Small non-motile rod</td>
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<td>Small non-motile rod</td>
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<td>Non-motile rod</td>
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<td>Non-motile rod</td>
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<tr>
<td><strong>Flagella</strong></td>
<td>Not observed</td>
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<tr>
<td><strong>Surface colonies on a gelatine plate</strong></td>
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<td>Same as I</td>
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<tr>
<td><strong>Gelatin slope</strong></td>
<td>Delicate thin growth with denuded margin</td>
<td>Delicate thin growth with denuded margin</td>
<td>Delicate thin growth with denuded margin</td>
<td>Delicate thin growth with denuded margin</td>
<td>Delicate thin growth with denuded margin</td>
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<td>Delicate thin growth with denuded margin</td>
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<tr>
<td><strong>Agar slope</strong></td>
<td>Moist grey growth</td>
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<tr>
<td><strong>Broth</strong></td>
<td>Diffuse growth</td>
<td>Same as I</td>
<td>Same as I</td>
<td>Same as I</td>
<td>Same as I</td>
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<tr>
<td><strong>Milk at 37° C</strong></td>
<td>No clot</td>
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<tr>
<td><strong>Peptone and salt solution</strong></td>
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<td>No indol</td>
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<tr>
<td><strong>Potato at 37° C</strong></td>
<td>Colourless growth</td>
<td>Colourless growth</td>
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<tr>
<td><strong>1% Glucose-peptone at 37° C</strong></td>
<td>N.G. Acid, 4%</td>
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<td>N.G. Acid, 4%</td>
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<tr>
<td><strong>1% Lactose-peptone at 37° C</strong></td>
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<td><strong>Saccharose-peptone at 37° C</strong></td>
<td>Idem</td>
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<tr>
<td><strong>1% Mannite-peptone at 37° C</strong></td>
<td>Idem</td>
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<td><strong>1% Galactose-peptone at 37° C</strong></td>
<td>Idem</td>
<td>Idem</td>
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<tr>
<td><strong>Proskauer's &amp; No. 1</strong></td>
<td>No growth</td>
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<td>No growth</td>
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<tr>
<td><strong>Capable's Media No. 2</strong></td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
<td>No growth</td>
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<tr>
<td><strong>Nitrate broth</strong></td>
<td>Reduces</td>
<td>Unchanged</td>
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<td>Unchanged</td>
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<td><strong>Neutral-red glucose</strong></td>
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<tr>
<td><strong>Anaerobiosis</strong></td>
<td>Growth</td>
<td>Growth</td>
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acidity being in all cases estimated after twenty-four hours' incubation at 37° C., unless specifically stated as being otherwise. The Proskauer and Capaldi media reactions are twenty-four-hour statements after incubation at 37° C.; indol production, clotting of milk, reduction of nitrate broth and neutral red reactions were all noted at the end of seven days' incubation at 37° C.

An examination of the table shows that while all this group of micro-organisms have certain common characteristics in respect of morphology, growth on agar, gelatine, in broth, in milk, on potato, in nitrate broth, and in Proskauer and Capaldi's media, it is in their behaviour in the various sugars and in peptone and salt solution that certain points of difference show themselves. All of these organisms ferment glucose with the formation of acid, and all fail to ferment lactose and saccharose, but in respect of some of the other sugars and the production of indol in Dunham's solution, they practically divide themselves into those which ferment one or more of the three sugars, maltose, mannite and galactose, and those which do not, and those which produce indol and those which fail to do so. A further scrutiny of the reactions shows that ability or inability to ferment one or more of these three carbohydrates coincides broadly with an ability or inability to produce indol in a peptone solution. A still closer analysis of the reactions indicates that we have apparently two groups of micro-organisms: one represented by all the Shiga bacilli, both the Kruse strains, Flexner I. and III., Harris, Bruce and the Vaillard variety, all fermenting glucose only; and a second group, represented by the Flexner II. and IV., Gray, Pickering and Landon; these organisms split dextrose, maltose, mannite and galactose, with the production of acid. In the main these results in the sugars are in accord with the work of Drigalski, Lentz and some others who have examined dysentery micro-organisms, but there are certain discrepancies, and an explanation of these discrepant results may be found in the fact that several of those observers have failed to see that the medium to which they have added carbohydrate such as lactose, mannite, &c., is glucose free. Most of their work has been done with bouillon, and no mention is made of the removal of muscle sugar from the broth used. This is a most important precaution, and in
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all my reactions care has been taken to see that there has been no trace of either glucose or muscle sugar.

As in the case of some other diseases, the blood serum of persons affected with dysentery appears to contain specific agglutinins, which are definitely active in respect of the infecting micro-organisms, and variably so in respect of other strains of apparently the same bacillus. This fact has naturally been taken to constitute an important argument in favour of the specific nature of the isolated micro-organism. Considerable time has been devoted to a study of the agglutination reactions of the bacilli actually isolated at Netley, as well as of those other cultures obtained from the various specified sources. The human blood serum was necessarily limited to that obtainable from the two cases which alone have come under observation, but in addition to these sera, some anti-colon serum was obtained from Dr. Dowson, of the Wellcome Research Laboratories, and various specific sera were obtained from rabbits inoculated with strains of the bacillus for the purpose. In all cases control observations were made with suspensions of B. coli and B. typhosus, also with an active anti-typhoid serum, while not infrequently normal human and rabbit’s blood was used as a control for the specific sera. In none of these control tests was an agglutination reaction ever obtained. The reaction was in all cases observed microscopically in a hanging drop, the time limit being placed at one hour, and no agglutination accepted as being positive if the dilution was less than 1 in 20. The results obtained may be summarised in these terms.

The blood serum from both Pickering and Landon failed to agglutinate any strain of the bacillus, even failing in respect of the apparently specific organisms isolated from their respective dejecta. A rabbit inoculated repeatedly with small doses of Landon’s bacillus gave a serum which agglutinated Landon 1 in 60, Flexner I. at 1 in 50, Shiga I. at 1 in 70. It failed to affect Pickering or Flexner II., or Harris and Gray. The other strains were not then available.

A rabbit inoculated with Flexner I. gave a serum which agglutinated Pickering 1 in 30, Landon and Kruse I. each at 1 in 50, Flexner I. at 1 in 80, but failed to touch Shiga I. or Kruse II. No other strains were available at this time.
A rabbit inoculated with Shiga I. was gradually worked up to agglutinate its own bacillus, and Flexner I. at 1 in 80, but had no effect on either Pickering, Landon or Flexner II. The action on Gray and Harris was doubtful even at 1 in 30.

A poly-valent anti-colon horse serum, obtained from Dr. Dowson, was efficient against Shiga I. at a dilution of 1 in 40, Flexner I. at 1 in 100, Flexner II. at 1 in 80, Gray, Harris and Landon at 1 in 60, Pickering and Bruce G. at 1 in 30. A second sample of anti-colon horse serum, kindly given me by Dr. Dowson, practically failed to touch any one of the various strains of dysentery bacilli.

The serum of a rabbit, as the result of inoculation with Shiga II., ultimately agglutinated its own bacillus at 1 in 110, Kruse I. and Shiga I. at 1 in 90, Flexner I. and III. at 1 in 80, Harris, Gray and Pickering at 1 in 40, but failed to act upon Landon, Flexner II. and IV.

A rabbit inoculated with Kruse I. gave a serum agglutinating that organism and Kruse II. at a dilution of 1 in 80, Flexner II. and III., also Harris, at 1 in 60, and Shiga I. at 1 in 40. It did not affect Flexner I., Pickering, Landon or Bruce G.

The serum from a rabbit as the result of inoculation with Kruse II. was only active against its own bacillus, Kruse I., all the Shigas and the Vaillard at 1 in 100. It was inactive against all the others.

The inoculation of a rabbit with Bruce G. gradually gave a serum active as to itself 1 in 100, Flexner II. and III. 1 in 40, but failed to touch either of the Shiga, the Kruse, Flexner I., or Pickering and Landon.

The serum from a rabbit which ultimately died from the effects of injections with Vaillard’s bacillus agglutinated its own micro-organism at 1 in 40, Shiga II. and Kruse II. both at 1 in 50, and Flexner IV. and Gray at 1 in 30. It was not tried against the other strains. Another rabbit, which had been inoculated with certain soluble toxins (to be referred to later) derived from Shiga’s bacillus, gave a serum agglutinating at 1 in 200 the Flexner, Kruse, Vaillard varieties, but affecting Shiga strains only at 1 in 80. It did not react on Bruce G.

These results leave little doubt as to the close identity of
the several bacilli with which the various observations have been made. At the same time it is clear that while the agglutinating properties of the bacilli from different dysenteric sources are very much the same, still there are differences in degree. Certain strains react better with a given serum than do others, especially if relatively high dilutions are used. Many of the sera were undoubtedly weak, and possibly this may explain why certain varieties of bacilli refused to react. The rabbit is not an ideal animal from which to obtain serum for this purpose, the goat and the horse being better; but the circumstances under which the inquiry had to be made did not permit of the use of these animals. Apart from this, the irregular agglutination phenomena noted as the result of attempts to obtain active sera after immunisation may be due to the formation of secondary agglutinins. Certain observations of Posselt and Segasser show that if one exhausts an agglutinating serum, by the addition to it of various micro-organisms, the absorption or fixing of agglutinins is not strictly specific, inasmuch as the immunising micro-organism absorbs not only the specific agglutinins, but also at times the secondary agglutinins or those specific to allied bacteria. At other times these secondary agglutinins may even increase instead of remaining stable or diminishing after the absorption of the specific agglutinins by the specific micro-organism. As explanatory of some of the difficulties surrounding the behaviour of dysenteric sera, Shiga has suggested the existence of certain hindering substances, or pro-agglutinoids, in an immunised serum which need to be fixed by the specific organism before the specific agglutinins can attach themselves.

Whatever may be the true explanation of the phenomena, an impartial review of this section of the inquiry clearly indicates that the formation of specific agglutinins, certainly in rabbits, as the result of gradual inoculations with dysenteric bacilli is both variable and feeble, and too much importance must not be placed upon either negative or positive agglutination reactions. Experience with the human subject accords with this view, as the specific agglutination reaction with the serum from persons suffering from dysentery can only be obtained usually within the first two weeks following the onset of acute symptoms, and even then is often slight. As will be seen
from what follows, there is much reason to believe that infection with bacilli of this group is not of the nature of a general septicemia involving a constitutional reaction, but rather of the nature of a local cytolysis. If this be the case, then the absence of specific agglutinins from the general circulation is intelligible.

Whilst fully admitting the value of the constant presence of the bacillus in the stools of persons suffering from dysentery, and of the fact that it is usually agglutinated by the serum of the affected person, as evidence of its specific pathogenicity, still the crucial test depends upon the ability to produce the disease experimentally by means of pure cultures of the microorganism. Until the publication of Vaillard's results at Vincennes the experimental facts have been somewhat ambiguous. As to the pathogenic effects of this bacillus upon man, the evidence is necessarily somewhat small, but even on this point it is not absolutely wanting. Strong reports the case of a Filippino prisoner, under sentence of death, who was induced to swallow a culture of the dysentery organism isolated in Manilla. The man suffered from the usual symptoms. The value of this case is somewhat lessened by the fact that dysentery was epidemic at the time. Shiga had one-twelfth of an agar culture, suspended in bouillon and killed by heat, injected into his own back. The immediate results of the injection were pain in the head, slight chill and fever. After six days, beyond the formation of a small abscess at the point of inoculation, the toxic symptoms passed off. Shiga's blood serum, however, showed active agglutination of the bacillus ten days after the injection. Flexner relates the experience of one of his laboratory assistants in Baltimore, who, while manipulating the bacillus from Manilla, accidentally aspirated a small quantity of fluid culture into his mouth. Notwithstanding immediate expectoration and free lavation of the mouth with a weak disinfectant, severe diarrhoea, with bloody and mucous stools, pain and tenesmus, developed within forty-eight hours.

On animals a considerable number of experiments have been made by numerous observers, but owing to the extreme susceptibility of all the ordinary animals to the toxins of the dysentery bacillus, lesions of the intestines analogous to those occurring in man, when suffering from the disease, have been
but rarely satisfactorily demonstrated. That they do result, however, has been successively maintained by Shiga, Flexner, Kruse, Vaillard and others, who report having caused marked enteritis, affecting chiefly the colon, in which the mucous membrane was oedematous, congested and beset with superficial necroses. The most emphatic lesions appear to have been produced in the dog, in the cat, in young pigs and rabbits. The horse reacts strongly to inoculations with the dysentery bacillus and not infrequently is killed by a small dose. The goat is more resistant, but rats, mice and guinea-pigs are quickly and easily affected, dying apparently from acute toxæmia. The channels of experimental infection have been various. Ingestion with food and by an oesophageal tube, even after preliminary neutralisation of the gastric contents or exhibition of an irritant, have not been successful, while even the direct introduction of pure cultures into the small intestine after laparotomy in a dog produced no appreciable effect. Intravenous injection of small quantities of a culture kills rabbits, dogs and small pigs in a few hours without allowing any definite lesion to be established. In large doses even subcutaneous injection is fatal to most animals, and in the rabbit, dog, cat and pig produces paralysis of the hind legs, with variable effects on the large intestine.

This aspect of the question has been the subject of considerable personal inquiry, and the following details furnish the results of my experiments as to bacillary dysentery in the rabbit. Various rabbits have not only been fed for days with cabbages soaked in fresh cultures of various strains of these dysentery bacilli, but also had considerable quantities passed into their stomachs by means of an oesophageal tube, but in no cases have the ingestion of the bacilli given rise to any symptoms or signs of ill-health. Subcutaneous injections of emulsions in sterile water of twenty-four hours old living growths on agar slopes of various strains of these bacilli have given rise to variable effects according to the particular strain employed and the dosage. Injections of the whole of a twenty-four hours old agar slope culture, emulsified in sterile water, of the different strains of Flexner II. and IV., Pickering, Landon and Gray, while producing certain agglutination functions on the blood serum, practically gave rise to no intestinal disturb-
Dysenteric ulcers in cæcum of rabbit.

Dysenteric ulcers in large bowel of rabbit.

Illustrating paper by Lieut.-Col. Firth.
ance or lesion or any symptoms other than a temporary fall in temperature of about 1°, lasting for a couple of days. On the other hand, injections of the Shiga, Kruse, Vaillard, Harris, Flexner I. and III., and Bruce strains produced serious disturbance. In doses of a sixth to a fourth of a twenty-four hours' growth on an agar slope, of the first six strains there was merely a temporary fall in temperature, following an initial rise, but in larger doses, varying from one-third to three-fourths of an agar slope culture, according to the size and weight of the rabbit, after an initial rise in temperature there was a marked lowering of body heat, with paralysis of the hind legs, progressive enfeeblement, and death about the fourth or fifth day. At no time was there actual diarrhœa, but on post-mortem examination the small intestine was generally found to be filled with a glairy mucus and the mucous membrane somewhat oedematous or thickened. The large bowel was usually found to be tumefied or oedematous, with here and there congested or haemorrhagic patches, while commonly in the caecum necrotic patches, involving large areas of mucous membrane, leading to local sloughs and ulcers, were to be found. The photographs of some of these specimens are shown in Plate II. Sections made of the diseased patches of the intestinal wall showed an extensive bacterial invasion of the mucous layer, the bacilli being generally disseminated, but in places collected in clumps or masses. Cultures made from the heart's blood, spleen, kidneys, liver and mesenteric glands invariably gave negative results. Sections made of the liver, spleen and kidneys failed to show any bacilli in those viscera, but sections of mesenteric glands indicated in a few instances their invasion with bacilli.

The effects following the subcutaneous injection of the strain Bruce G. into a rabbit were slightly different from those caused by other varieties of this bacillus. Its action was slower, the animal on the seventh day being seized with a severe diarrhœa, passing liquid motions with mucus and slimy matter. An autopsy of the animal showed extensive enteritis involving the lower part of the small bowel, the caecum and the upper part of the large intestine. No distinct ulcers were noted, at most some haemorrhagic patches, but the whole mucous surface of the affected bowel was acutely inflamed as if by some violent irritant.
Cultures from the viscera and blood and sections of the affected parts were negative.

The intestinal lesions produced in the rabbit by subcutaneous injection of these dysentery bacilli bear a striking resemblance to those characteristic of the disease in man. The most remarkable fact is, however, the selective affinity which the micro-organism displays for a single viscera, viz., the cæcum and large intestine, directing upon their tissues all its effects, and apparently not touching any other organs. At the point of inoculation there results a temporary tumescence, and marked by a slight local development of the injected bacilli, but this is evidently but transitory, the micro-organisms either apparently getting into the blood-stream and quickly finding their way to the elective tissues where their toxic action rapidly produces necrotic effects; or undergoing bacteriolysis in the blood, their liberated toxins gradually exert a specific action on the intestinal mucous membrane.

The marked effects which result from the injection of living cultures of certain strains of these bacilli suggest the question, what results are produced by these bacilli (1) if killed by heat, (2) if killed by some chemical reagent, such as chloroform, and (3) do these bacilli secrete or contain a toxin affected by heat? These various points have been investigated with the following results:—

The dysentery bacilli are killed by an exposure to 60° C. for half an hour. Intravenous or hypodermic injections of emulsion of dead bacilli of the Kruse, Shiga, Vaillard and Flexner I. and III. strains, which had been killed by an exposure to this temperature, gave rise in rabbits to symptoms similar to those produced by the corresponding living cultures, the essential difference being that the fatal effect was somewhat delayed. If a culture of these bacilli on an agar slope be killed by exposure to chloroform vapour, and the dead bacilli be worked up into an emulsion in sterile salt solution, a fatal effect in rabbits, with intestinal lesions in the cæcum and large bowel, follows a subcutaneous injection in the back about the fourth day, marked also by hypothermia and paralysis of the hind legs.

The dysentery bacilli appear to secrete a soluble toxin, at least rabbits succumb to hypodermic injections of 5 cc. of the sterile filtrate from a four-day-old broth culture incubated at
37° C., obtained by filtration through a porcelain filter. The resulting symptoms during life and effects on the agglutinating powers of the blood serum are practically identical with those produced by injections of living bacilli, while the post-mortem appearances are mainly necrotic changes in the mucous membrane of the cæcum and large bowel. Fig. 1 shows a sloughing patch from the large bowel of a rabbit following a subcutaneous injection of 5 cc. of a sterile filtrate from a four-day-old broth culture of Shiga's bacillus. This toxic material, apparently secreted by the bacilli into broth in which they are growing, is no longer pathogenic to rabbits after an exposure to 65° C. for half an hour, but injections of this now no longer toxic material is still capable of setting up specific agglutination power in the blood serum, and also rendering the anima distinctly refractive to lethal doses of living dysentery bacilli. This suggests the existence of two bodies in this material, namely, a labile toxic and a stable functional substance.

A similar but more powerful toxic substance can be dissolved out of the dead bacilli by water. Following the lines suggested by the recent work of Conradi, Neisser and Shiga, six agar slope cultures of Vaillard's bacillus were incubated at 37° C. for twenty-four hours. These cultures were then killed by an exposure to chloroform, the dead bacilli were then scraped off

Fig. 1.—Piece of sloughing mucous membrane from large bowel of rabbit.
the agar and worked up into an emulsion with 50 cc. of sterile salt solution. This was allowed to autolyse for fourteen days at 37° C. The emulsion was then centrifugalised and the supernatant clear liquid pipetted off and filtered through a porcelain filter. An intravenous injection of 1 cc. of this sterile liquid into a rabbit weighing 2-1 kilogrammes caused death in seventeen hours. A subcutaneous injection of 2 cc. into a rabbit of similar size caused hypothermia, paralysis of the hind legs, and some diarrhoea. On being killed, under chloroform, an examination of the body showed extensive ecchymoses and superficial necrosis of the mucous membrane of the caecum. In fact, similar lesions to those caused by injections of the living bacilli and their toxins secreted into broth. Some of this aqueous extract of dead bacilli was heated to 65° C. for half an hour: its toxicity was not sensibly lessened when hypodermically injected into a rabbit. Exposure to 75° C. for half an hour rendered it no longer toxic. Similar observations were made with the toxins dissolved out of dead bacilli of the Shiga strains.

On analogous lines to some work done by Carega in respect of B. coli, the following observations were made as to the active substances contained in the bodies of one of these strains of dysentery bacilli; 500 cc. of broth were inoculated with Shiga II. and incubated at 37° C. for sixteen days. Over a water bath at 50° C. the culture was reduced to half and the contained proteids precipitated by the addition of 200 cc. of absolute alcohol. The precipitate was collected and digested in 50 cc. of 0.5 per cent. solution of caustic soda for twenty-four hours. After filtration the precipitate was dried at 40° C. This substance was set aside as being presumably a nuclein. The clear alkaline filtrate was then acidified with acetic acid, and the resulting precipitate, collected on a filter, was washed several times with very dilute acetic acid and finally dried at 40° C. This substance was deemed to be a nucleo-albumin. Of the nuclein there was 0.11 gramme and of the nucleo-albumin 0.08 gramme. The nuclein was rubbed up with sterile water and sterilised at 60° C. on three successive days; 0.005 gramme injected into the ear vein of a rabbit caused death within a quarter of an hour; given hypodermically, in a dose of 0.02 gramme, it caused at first some local irritation; on repeating
the dose every three days a cumulative toxic effect was produced, the animal dying after the third dose. No appreciable lesions were noticeable, nor was the blood rendered capable of agglutinating any dysentery bacilli. The toxicity of this nuclein was not sensibly diminished after half an hours' exposure to 65° C. The nucleo-albumin, on the other hand, had feeble toxic powers, though it was capable of establishing some specific agglutination reaction in the serum. This function was not absolutely nullified by an exposure to 65° C. for half an hour. There was no evidence of any immunising power. Taken in conjunction with the facts mentioned on page 451, these observations are not without interest.

Recognising the apparent elective affinity which the pathogenic strains of these bacilli have for certain parts of the intestinal tract, it appeared desirable to determine whether there was any evidence of the existence of neutralising substances for the toxins of these bacilli in any tissues of the rabbit's body. For this purpose, a healthy rabbit was selected and killed under chloroform. The spleen, some liver tissue, kidney, voluntary muscle, and portion of caecum and large intestine were rapidly removed and placed in sterile normal salt solution at 37° C. The tissues were rapidly washed in the saline and two grammes each of spleen, liver, kidney and voluntary muscle rubbed up in a sterile mortar with an emulsion made from a twenty-four hour agar slope of Shiga's bacillus. The portion of bowel removed measured some ten inches in length: the mucous surface was carefully scraped with the back of a sterile knife, so as to practically remove all the mucous membrane. This emulsified material, measuring about 3 cc., was now rubbed up with an emulsion in saline solution made from the scraped surfaces of a twenty-four hour agar slope of Shiga's bacillus. All the mixtures were transferred to sterile test-tubes and incubated for two hours at 37° C. They were then centrifugally and 5 cc. of each of the clear supernatant fluids injected subcutaneously into five rabbits, a sixth being taken for a control injection. The results were as follows: The control died on the third day, the liver, spleen, kidney and voluntary muscle animals all died on the fourth day, the symptoms and post-mortem appearances being similar to those already described. The animal which received the emulsion of bacilli and intestinal
mucous membrane survived till the seventh day, when owing to the onset of posterior paralysis it was killed. The post-mortem lesions were small hemorrhagic necroses in the cæcum. A second experiment, on similar lines, indicated in even a more marked manner the antitoxic influences of the intestinal epithelia.

So far as it goes, this series of experiments clearly suggests the existence of some fixing or neutralising substance in the epithelia of the intestinal tract of the rabbit. That such is probably the case is supported by the difficulty to secure infection by ordinary feeding with dysentery bacilli and by the following observations. Laparotomy was performed on two rabbits; into the cæcum of one was injected by means of a fine needle an emulsion, made in sterile water, of two twenty-four-hour-old agar slopes of Vaillard's bacillus, while into the bowel of the other 20 cc. of freshly filtered broth from a four-day-old bouillon culture of Shiga's bacillus were injected. In neither case was there the slightest evidence of discomfort or illness. A week later, on the rabbit which had received the soluble toxins a second laparotomy was performed, and an emulsion of living bacilli from two twenty-four-hour-old agar slopes of the same strain injected into the cæcum. At the end of a fortnight the animals were killed; the intestinal mucous membrane in both was quite normal. It will be remembered that smaller doses of the same culture and filtrate when given subcutaneously were markedly pathogenic.

Arising, naturally, from these experimental observations as to the specific action of these bacilli is the question of securing immunity from their effects. My own experiments relating to this topic have been few and the results are too indefinite to justify extended notice. They are, however, sufficiently encouraging to warrant further work, which necessarily will furnish the subject of a future communication. The same question has been approached by other workers, notably by Shiga and Gay. The former has produced a serum by immunising goats with cultures killed by heat which he reports to be both protective and curative. He employed it in an extensive epidemic in Japan, with a death-rate of 9·6 per cent., while the mortality during the same period in the same epidemic under ordinary treatment was 34·7 per cent. Gay's work has
been purely an experimental study on guinea-pigs, immunised with dead cultures against lethal doses of the living bacilli, and also covers inquiries as to the protective power of immunised horse serum on the same animals against living cultures of *B. dysenteriae*. He finds that a vaccine made from dead bacilli is sufficient to protect guinea-pigs from a succeeding multiple fatal dose of living dysentery bacilli, and to produce in the horse an active immune serum. This immune serum of the horse exhibits marked protective properties on guinea-pigs against fatal infection with *B. dysenteriae* or its toxin. A useful serum therapy of bacillary dysentery is, therefore, rendered highly promising.

Further important points in relation to this group of microorganisms are, what is their ability to survive outside the human body, and what technique is best adapted for their isolation. In respect of their viability, certain personal observations indicate that when dried on pieces of rag or placed in dry soil the dysentery bacillus will remain alive from twelve to twenty-two days, according to the temperature, the former figure being obtained when the room temperature was 12° C., and the latter when it was 28° to 35° C. When placed in ordinary tap-water it is recoverable up to twenty-five days at 22° C., but if the water be kept in the incubator at 37° C. it was recoverable on the thirty-sixth day. From sterile water stored at room temperature of 14° C. it was recoverable up to forty-three days. When spread on breadcrumbs it survived six days. The isolation of dysentery bacilli in the early stages of a case is by no means difficult, but quite otherwise when the acute symptoms pass off. Probably the best technique to adopt is to inoculate one or more broth tubes, or tubes containing sterile water, with loopfuls from the mucus which is so characteristic of dysenteric excreta. It is futile dipping the needle or loop into the feculent mass. After inoculation incubate for twelve hours at 37° C., and then from these tubes streak, with concentric rings, the surfaces of a series of neutral litmus lactose agar plates as already explained on page 398 of this Journal, using one needleful for streaking at least three plates before reinoculating. Owing to the dysentery bacilli having no effect on lactose their resulting colonies will appear as translucent glistening blue points, while all the colonies
of colon bacilli and others which produce acid on this sugar will be red. The absolute recognition of the identity of the isolated micro-organisms, in the absence of an active specific serum, is dependent upon careful subculturing, in accordance with details already discussed.

In attempting to summarise this somewhat imperfect study, it seems permissible to draw the following conclusions:

(1) That the various strains of Shiga, Kruse, Vaillard, Harris, and Flexner I. and III. are practically identical. Bruce G. resembles them closely culturally, but produces perhaps less alkali in milk, and also gives colonies in gelatine which are more plicated than the others. It also fails to respond definitely in agglutination reactions to sera which are active to other strains. Pathologically its action is slightly different, at least in the rabbit. Too much importance must not be attached to these differential features, as the culture of the micro-organism in question was nearly dead when brought to this country, and had to be reactivated by a succession of sub-cultures. The other strains, Flexner II. and IV., Gray, Pickering and Landon, are the same, but apparently non-pathogenic to rabbits. These latter varieties present some cultural differences in the sugars from the other strains examined.

(2) That in the intestinal dejecta of acute dysentery cases bacilli can be found which have certain characteristics differentiating them from the common colon bacilli and the micro-organism of enteric fever. These bacilli are agglutinated only by the blood of men or animals either suffering from epidemic dysentery, or infected by them and their elaborated toxic substances.

(3) By the subcutaneous inoculation of some of these bacilli, or of their contained and excreted toxic substances, one can produce in rabbits symptoms and intestinal lesions characteristic of epidemic dysentery in man.

(4) The toxic substances elaborated by or contained in the bodies of these bacilli have a selective affinity for the mucous membrane of the cæcum and large bowel. But owing to the apparently high refractiveness of the epithelial lining of the intestinal tract of rabbits to these bacilli and their toxins, the production of intestinal lesions or general infection by them in these animals is not possible either by ordinary methods
of ingestion or direct introduction into the various parts of their alimentary canal.

(5) That the excreta of dysentery cases contain not only pathogenic bacilli of definite cultural characters, but also others which, apparently, are not pathogenic, though presenting certain superficial cultural resemblances. So far as this present inquiry goes, the distinctive feature of the non-pathogenic group seems to be an ability to split maltose, mannite and galactose, to the formation of acid, but without production of gas, also to produce indol, characteristics which are wanting in the pathogenic varieties. These non-pathogenic forms are probably the "pseudo-dysentery bacilli" of some authors, but whether they represent degraded or transitional forms of the pathogenic type we are not in a position to say.

(6) The clinical entities produced by the pathogenic dysentery bacilli probably represent a group rather than a single class of cases. The cases may range from the typical acute dysentery of camps, through the various degrees of ileo-colitis, to the infective diarrheas of infants and adults. The causative agent in these cases is probably a micro-organism corresponding to one or other of the pathogenic types described in this article. Although there is much to warrant a belief that an acquired immunity may be secured against individual varieties of dysentery bacilli, the mere fact of there being a possible plurality of varieties of these bacilli, capable of causing a train of clinical symptoms common to them all, suggests great difficulties in the way of producing a condition of general immunity against infection by the whole group.

In concluding this article, I desire to thank my colleague, Captain Fowler, for much aid in the various cultural manipulations and autopsies of animals examined, also Major Leishman and Captain D. Harvey for kindly preparing and cutting sections. As a considerable number of references have been made to the literature of this subject, already of some extent, a short bibliography is appended which may be of use to others wishing to look up the papers in their original form.

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